Ecological Indicators 95 (2018) 1026–1037

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

Original Articles

Heat wave impacts on the model diatom *Phaeodactylum tricornutum*: Searching for photochemical and fatty acid biomarkers of thermal stress

Eduardo Feijão[a], Carla Gameiro[b], Marco Franzitta[b], Bernardo Duarte[b], Isabel Caçador[b], Maria Teresa Cabrita[c,1], Ana Rita Matos[a,*]

[a] *BioISI—Biosystems and Integrative Sciences Institute, Plant Functional Genomics Group, Departamento de Biologia Vegetal, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal*
[b] *MARE – Marine and Environmental Sciences Centre, Faculty of Sciences of the University of Lisbon, Campo Grande, 1749-016 Lisbon, Portugal*
[c] *Portuguese Institute of Sea and Atmosphere (IPMA), Av. de Brasília, 1449-006 Lisboa, Portugal*

ARTICLE INFO

Keywords:
Eicosapentadienoic acid (EPA)
Omega-3 fatty acids
Photosynthesis
Biomarkers
Phaeodactylum tricornutum
Climate change

ABSTRACT

Global warming is increasing the frequency and intensity of extreme thermal events, with inevitable consequences for marine ecosystems and organisms. Phytoplankton is at the base of marine food webs and diatoms are major contributors to global primary production. Therefore, environmental changes, such as heat, influencing growth, physiology and biochemical composition of diatoms, impact other organisms at higher trophic levels. The model diatom *Phaeodactylum tricornutum*, particularly rich in the long chain omega-3 eicosapentaenoic acid (EPA), and able to accumulate substantial amounts of storage lipids, has recently been the object of numerous works, regarding fundamental aspects of lipid metabolism and exploring its biotechnological potential for biodiesel and aquaculture purposes. The aim of this study was to use *P. tricornutum*, growing under controlled conditions, to examine the effects of a heat wave, in order to identify heat stress biomarkers. The photosynthetic and respiratory metabolism was investigated by Chlorophyll *a* fluorescence and by O_2 evolution and discussed in connection with changes observed in the composition of photosynthetic pigments and fatty acids. *Phaeodactylum tricornutum* cells exposed to 26 °C displayed lower photosynthetic O_2 production, but similar respiratory rate, comparing to cells at control temperature (18 °C), which is likely related to the biomass decrease observed under heat stress. Heat wave exposed cells also showed a less efficient PSII, higher energy dissipation and higher chlorophyll *a* and fucoxanthin concentrations, suggesting a heat-induced amplification of the light energy absorption capacity. Heat wave exposed cells showed lower relative EPA contents and double bond indexes, whereas the parameter inversely related to nutritional value, omega 6/omega 3 ratio, increased. Moreover, the analysis of the fatty acid profiles also suggested that heat exposure negatively impacted thylakoid lipids, in agreement with the decrease observed in photosynthesis. Results obtained highlight the negative impact of heat waves on diatom photosynthesis and nutritional value, as well as on their capacity to oxygenate ocean water. Furthermore, physiological parameters as well as fatty acids and photosynthetic pigments signatures, were identified, that could represent expedite biomarkers of thermal stress in future studies.

1. Introduction

As defined by the World Meteorological Organization (WMO), a heat wave refers to an event that lasts at least five consecutive days, with maximum daily temperature exceeding the average maximum temperature by 5 °C, for the 1961–1990 reference period. Although heat waves are most directly felt in terms of air temperature, they are also linked to a rise in sea surface temperatures (Wernberg et al., 2012). The IPCC WG2 5th Assessment Report (IPCC, 2014) points to an enhancement of global air temperature, and to a further increase in the frequency and duration of extreme heat events. While cold waves might decrease in frequency, heat waves appear to be gaining in intensity. Over the past 10 years, Mediterranean countries have experienced heat with higher frequency (Niu et al., 2014), with the total number of heat wave days dramatically increasing and long-lasting heat waves prevailing (Baldi et al., 2005). Since the 1980s, warming has been stronger in the Iberian Peninsula, mostly during summer (EEA, 2012; Dasari et al., 2014) and surface water temperature in the Tagus estuary

* Corresponding author.
E-mail address: armatos@fc.ul.pt (A.R. Matos).
[1] Present affiliation: Centro de Estudos Geográficos (CEG), Instituto de Geografia e Ordenamento do Território (IGOT), University of Lisbon, Rua Branca Edmée Marques, 1600-276 Lisbon, Portugal.

http://dx.doi.org/10.1016/j.ecolind.2017.07.058
Received 5 March 2017; Received in revised form 21 July 2017; Accepted 28 July 2017
Available online 10 August 2017
1470-160X/

reached 26 °C during the particularly hot summer of 2005 (Gameiro et al., 2007).

The rise in seawater temperature caused by these extreme climatic events can dramatically affect marine organisms, populations and ecosystem functions (Harley et al., 2006; Doney et al., 2012). In particular, changes in the physiology of marine organisms can occur (IPCC, 2014) inducing alterations, for instance, in the biomolecules content, with repercussions to the marine food webs (Galloway and Winder, 2015). Effective biomarkers in model organisms allow to link harmful effects of heat wave stress in those species to their ecological consequences, providing evidence on the health status of organisms, populations and ecosystems. In coastal areas, where increasing heat wave events are becoming an enduring problem, the establishment of effective marine biomonitor species equipped with efficient biomarkers as key-tools for marine ecological assessment is highly required.

Phytoplankton are at the base of marine food webs and are also major producers of many complex biomolecules, including fatty acids in diverse lipid classes (Guschina and Harwood, 2009). Photosynthetic organisms are able to synthetize linoleic- and linolenic acids, which belong to the classes omega 6 (ω-6) and omega-3 (ω-3), respectively and are essential fatty acids (EFA) for vertebrates. EFA are precursors of long chain polyunsaturated fatty acids (LC-PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which play key roles in heart health, immune and inflammatory responses, visual acuity as well as being major components of neurological tissues such as the brain and spinal cord (see review from Wiktorowska-Owczarek et al., 2015). Since most organisms of higher trophic levels of marine food webs, such as fish and humans, have limited ability to produce LC-PUFA from EFA, they rather obtain them from the diet, relying on their *de novo* production by aquatic algae (Parrish, 2009; Arts et al., 2001). The classic "Western diet" is particularly imbalanced towards foods containing high ω-6/ω-3 ratios, such as meat and plant oils, contrasting to a low value in algae and marine fish (for a review see Mühlroth et al., 2013). Therefore, this parameter is an important determinant of the nutritional value of a particular food source. Also, the FA composition of cell membranes is a key factor allowing cells to deal with changes in environmental conditions (Upchurch, 2008). Because phytoplankton fatty acid composition is affected by temperature (Guschina and Harwood, 2009; Dalsgaard et al., 2003) heat waves can modify the fatty acid composition of phytoplankton, as well as their ω-6/ω-3 ratios, and cause alterations in the structure of marine food webs (Budge et al., 2014), with possible impacts on the fish stocks of commercial interest. Therefore, fatty acids have the potential to be efficient biomarkers of thermal stress in marine organisms (Filimonova et al., 2016) and thus acquire reinforced relevance in biomonitoring studies.

Elevated temperatures also affect the photochemistry of phytoplankton. Although phytoplankton are generally able to efficiently photosynthesise over a temperature range around the optimal growth temperature, high temperature may cause impairment in the photosynthetic electron transport and carbon fixation mechanisms leading to a decline in photosynthetic performance and efficiency (Falk et al., 1996). Together with fatty acids, phytoplankton photosynthetic responses to elevated temperature may be applied as potential biomarkers for heat wave assessment in marine environments (Xu et al., 2015). Coupling these biomarkers with efficient non-destructive methods, allows a quick assessment of the impacts of a certain stress, such as a heat wave, almost in real time. Pulse Modulated Amplitude (PAM) Fluorometry is one of these methods which evaluates the photonic energy harvest and transformation processes into electronic energy, using the correspondent chlorophyll a (Chl *a*) fluorescence signals as proxy. Any disturbance at the primary productivity level is efficiently reflected by PAM fluorometry (Anjum et al., 2016), able to capture the whole photochemical process and key steps in photon capture, energy transduction and dissipation. These tools have previously proved to be very useful to evaluate the effects of stress conditions on primary productivity giving important insights on its effects at the energetic transduction level (Duarte et al., 2015, 2016; Anjum et al., 2016).

In this study it was hypothesised that the fatty acid content and composition and photochemistry parameters in marine phytoplankton species, subjected to elevated temperature conditions, are sensitive and reliable biomarkers of thermal stress for the assessment of heat wave events in marine systems. Changes in fatty acids and photosynthetic parameters, triggered by elevated temperature in the model species *Phaeodactylum tricornutum*, were investigated in order to understand the effects of these extreme climatic events at the phytoplankton physiological level, and also to evaluate the efficiency of potential biomarkers to detect early stress signs of heat waves in coastal areas. Because diatoms are dominant phytoplankton species in the marine environment, able to reflect the early signs of stress at the ecosystem level (González-Dávila, 1995), the diatom *P. tricornutum* was chosen as biomonitor model species. This species is also widely used in bioassays to evaluate the impacts of various stressors to the phytoplankton community (De Martino et al., 2011), responding quickly to environmental variations (Cabrita et al., 2016). Furthermore, this diatom is tolerant to a wide temperature range (Bojko et al., 2013), and produces an extensive array of fatty acids, including high amounts of EPA. The aim of this study was to investigate efficient fatty acid- and photochemistry parameters as potential biomarkers of thermal stress that can be employed for the early detection of heat wave events in marine systems.

2. Materials and methods

2.1. Experimental set-up

Control axenic cultures of the diatom *Phaeodactylum tricornutum* Bohlin (Bacillariophyceae) (IO 108-01, IPMA) were maintained in 250 mL flasks containing f/2 medium (Guillard and Ryther, 1962), in a growth chamber (Fytoscope FS130), at temperature-controlled conditions (18 ± 1 °C), with constant aeration, during 5 days. The chamber was programmed with a sinusoidal function simulating sunrise and sunset, with a light intensity at noon to simulate a natural light environment (RGB 1:1:1, Maximum PAR 80 μmol photons $m^{-2} s^{-1}$, 14/10 h day/night rhythm). Initial cell concentration was approximately 2.7×10^5 cells mL^{-1}, following the Organization for Economic Co-operation and Development (OECD) guidelines for algae bioassays (OECD, 2002) and the therein recommended initial cell density for microalgae cells with similar size to *P. tricornutum*. In parallel, *P. tricornutum* cultures at the beginning of the exponential phase (48 h later) were grown as the control cultures, but subjected to a temperature of 26 ± 1 °C, mimicking the maximum water temperature registered by Gameiro et al. (2007) in the Tagus Estuary, during the 2005 heat wave. All materials used were cleaned with HNO_3 (20%) for two days and rinsed thoroughly with Milli-Q water (18.2 MΩ cm) and autoclaved to avoid contamination. Culture manipulations were performed in a laminar air flow chamber.

2.2. Cell growth rates

Samples of *P. tricornutum* of both control and elevated temperature exposure experiments were taken for cell counting on a Neubauer improved counting chamber, under a Olympus BX50 (Tokyo, Japan) inverted microscope, at ×400 magnification. Growth was estimated as the mean specific growth rate per day, calculated from the difference between initial and final logarithmic cell densities divided by the exposure period, as proposed by Santos-Ballardo et al. (2015). The number of cells aggregated in pairs or in groups of more than two (clamps) was also registered, in order to calculate their relative abundance. For determination of fresh weight and collection of samples for biochemical analysis, cells were collected at the end of the experiments, centrifuged at 4000*g* for 15 min at 4 °C and after supernatant removal, immediately frozen in liquid nitrogen and stored at −80 °C. Three biological replicates for each analysis were used for control (18 °C) and

elevated (26 °C) temperature exposed cell samples.

2.3. Pulse amplitude modulated (PAM) fluorometry

Pulse amplitude modulated (PAM) chlorophyll fluorescence measurements were performed using a FluoroPen FP100 (Photo System Instruments, Czech Republic), on samples using a 1 mL cuvette. Cell density was assessed daily for comparison purposes using a non-actinic light (Ft). All fluorometric analysis were carried out at the end of the experimental period in dark-adapted samples. Rapid Light Curves (RLC) were achieved using the pre-programmed light curve (LC1) protocol which performs successive measurements of the sample photosystem II efficiency (ϕPSII) under various light intensities (20, 50, 100, 200, 300 and 500 μmol photons $m^{-2} s^{-1}$) of continuous illumination relating the rate of photosynthesis to photon flux density (PAR). Chlorophyll transient light curves were also assessed by the OJIP test. In short, the level O represents all the open reaction centres (RC) at the onset of illumination with no reduction of quinone a (Q_A) (fluorescence intensity lasts for 10 ms). The rise of transient fluorescence from O to J indicates the net photochemical reduction of Q_A (the stable primary electron acceptor of PSII) to Q_{A-} (lasts for 2 ms). The phase from J to I was due to all reduced states of closed RCs such as Q_{A-} Q_{B-}, Q_A Q_{B2-} and Q_{A-} Q_B H_2 (lasts for 2–30 ms). The level P (300 ms) coincides with maximum concentration of Q_{A-} Q_{B2} with plastoquinol pool maximally reduced. The phase P also reflects a balance between light incident at the PSII side and the rate of utilization of the chemical (potential) energy and the rate of heat dissipation (Zhu et al., 2005). Table 1 summarizes all the parameters that were computed from the fluorometric data.

2.4. Oxygen evolution analysis

At the end of the experiments, the photosynthetic and respiratory oxygen production and consumption, were assessed, respectively, using 1 mL culture samples exposed to white light (LS2 light source) emitting 100 μmol photons $m^{-2} s^{-1}$ using a Clark-type electrode (S1 Electrode Disc, Hansatech Instruments, England) with constant mixing (Matos et al., 2009). Temperature was maintained at experimental growth conditions (18 °C and 26 °C) by a circulating water bath directly connected to the electrode chamber. Data analysis was made with OxygraphPlus software.

Table 1
Summary of fluorometric analysis parameters and their description.

Rapid Light Curves (RLC)	
rETR	Relative electron transport rate at each light intensity (rETR = QY × PAR × 0.5)
ETR_{max}	Maximum ETR obtained from the RLC after which photo-inhibition can be observed
α	Photosynthetic efficiency, obtained from the initial slope of the RLC
β	RLC related respiration, obtained from the final slope; Photo-inhibition parameter
JIP-test	
Area	Corresponds to the oxidized quinone pool size available for reduction and is a function of the area above the Kautsky plot
N	Total number of electrons transferred into electron transport chain
S_M	A measure of the energy needed to close all RCs
AOECS	Activated oxygen-evolving complexes
ABS/CS	Absorbed energy flux per cross-section
TR_O/CS	Trapped energy flux per cross-section
ET_O/CS	Electron transport energy flux per cross-section
DI_O/CS	Energy dissipated as heat per cross-section
RC/CS	Number of available reaction centres per cross section
P_G	The grouping probability is a direct measure of the connectivity between the two PSII units
M_O	Observed rate of Quinone A reduction
$ΨE_O$	Efficiency with which a PSII trapped electron is transferred from Q_A to Q_B

2.5. Pigment analysis

Cell sample pigments were extracted with 100% acetone and maintained in a cold ultra-sound bath for 2 min, to ensure complete disaggregation of the cell material. Pigments were extracted at −20 °C for 24 h in the dark to prevent degradation (Cabrita et al., 2016) and then centrifuged for 15 min at 4000*g* at 4 °C. The supernatants were scanned in a dual beam spectrophotometer from 350 nm to 750 nm at 0.5 nm steps. The absorbance spectrum was introduced in the Gauss-Peak Spectra (GPS) fitting library, using SigmaPlot Software. Pigment analysis was employed using the algorithm developed by Küpper et al. (2007). Thus, Chlorophyll *a* and *c*, Pheophytin a, β-carotene, Fucoxanthin (Fx), Diadinoxanthin (Ddx) and Diatoxanthin (Dtx) were detected. The De-Epoxidation State (DES) was calculated as:

$$DES = ([Dtx]/([Dtx] + [Ddx]))$$

2.6. Fatty acid analysis and quantification of lipid peroxidation products

Fatty acid analysis was performed by direct trans-esterification of cell pellets, in freshly prepared methanol-sulfuric acid (97.5:2.5, v/v), at 70 °C for 60 min, as previously described for higher plants leaves (Gameiro et al., 2016; Duarte et al., 2017) using pentadecanoic acid (C15:0) as internal standard. Fatty acids methyl esters (FAMEs) were rescued using petroleum ether, dried under a N_2 flow and re-suspended in an appropriate amount of hexane. One microliter of the FAME solution was analyzed in a gas chromatograph (Varian 430-GC gas chromatograph) equipped with a hydrogen flame ionization detector set at 300 °C. The temperature of the injector was set to 270 °C, with a split ratio of 50. The fused-silica capillary column (50 m x 0.25 mm; WCOT Fused Silica, CP-Sil 88 for FAME; Varian) was maintained at a constant nitrogen flow of 2.0 mL/min and the oven temperature set at 190 °C. Fatty acids were identified by comparison of their retention times with standards (Sigma-Aldrich) and chromatograms analyzed by the peak surface method, using the Galaxy software. The double bond index (DBI) and the ω-6/ω-3 ratios were calculated as follows:

$$DBI = 2\ [(\%\ monoenes) + (2\times\ \%\ dienes) + (3\times\ \%\ trienes) + (4\times\ \%\ tetraenes) + (5\times\ \%\ pentaenes)]/100$$

$$ω\text{-}6/ω\text{-}3 = (C18{:}3 + C20{:}4)/(C18{:}4 + C20{:}5).$$

Quantification of the lipid peroxidation products was performed as described before (Duarte et al., 2015), with minor modifications, such as homogenization in 10% (v/v) Trichloroacetic acid (TCA) and brief sonication. Absorbance values at 532 nm and 600 nm were registered and the concentration of TBARS calculated using the molar extinction coefficient, 155 $mM^{-1} cm^{-1}$ (Heath and Packer, 1968).

2.7. Statistical analysis

Due to lack of normality and homogeneity, the statistical analysis of the data was performed with a non-parametric test. Using the IBM SPSS Statistics 23 software to compare the effects of the temperature, the Mann-Whitney test evaluated significant differences between samples. The correlation between parameters was assessed with a Spearman correlation test. The Student's *t*-test was performed to compare the slopes of the 18 and 26 °C cell density versus basal fluorescence regression lines. Significance was assumed for both tests when p value ≤ 0.05.

3. Results

3.1. Growth parameters

Exposure to the temperatures, 18 and 26 °C, affected *P. tricornutum* biomass production, expressed in terms of cell density, specific growth

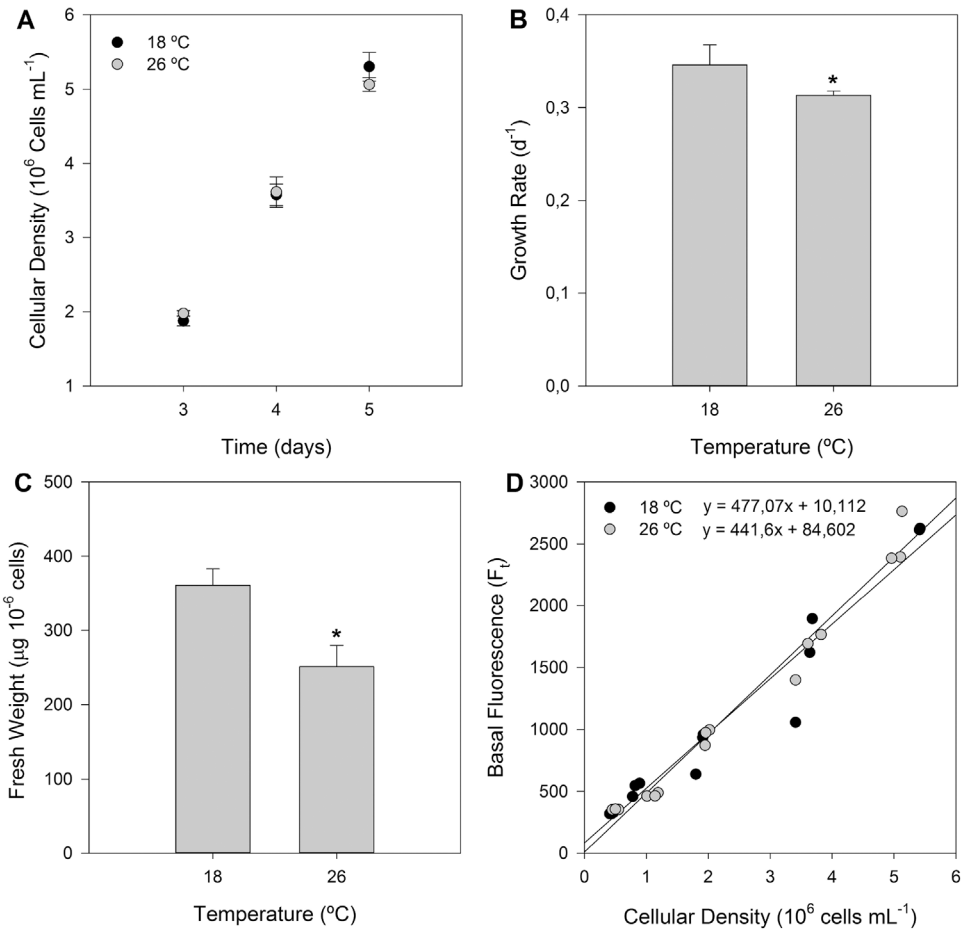

Fig. 1. Growth parameters of P. tricornutum grown at 18 °C (black) and exposed to a heat wave (26 °C, for three days) (gray). A) Cell density; B) Specific growth rate; C) Fresh weight; D) linear relationship between cell density and basal fluorescence. Values correspond to average ± standard error, n = 3; asterisks indicate significant differences ($p \leq 0.05$).

rate and fresh weight (Fig. 1). After three days of exposure to 26 °C, cultures showed a slightly lower cell number compared to cultures kept at 18 °C (Fig. 1A). Accordingly, growth rates were significantly lower ($p \leq 0.05$) for cells exposed to 26 °C in comparison to 18 °C grown cells (Fig. 1B). Fresh weight of cells exposed to 26 °C was significantly ($p \leq 0.05$) lower in comparison with values obtained for cells grown at 18 °C (Fig. 1C). Interestingly, elevated temperature promoted the formation of cell pairs, with a consequent decrease in cell clamps (pairs: 10%; clumps: 12%), contrarily to that observed in cells grown at 18 °C (pairs: 8%; clumps: 17%). Only fusiform cells of *P. tricornutum* were observed (data not shown).

In parallel to microscopy observation, cell density was also evaluated by PAM fluorometry. A highly positive correlation ($R^2 = 0.96$, $p = 0.0004$) between cell number and basal fluorescence (F_t) was observed for both 18 and 26 °C grown cells (Fig. 1D), and significant differences were detected between the slopes of the 18 versus 26 °C regression lines ($t(1)7 = 2.407$, $p > 0.05$).

3.2. Photosynthetic and respiratory traits

Rapid light curves of relative electron transport rates were performed at the end of the experiment and are depicted in Fig. 2. For photosynthetic electron transport rates at different light intensities, changes were notable in heat exposed cells, starting at 100 µmol photons $m^{-2} s^{-2}$ (Fig. 2A), being that ETRs values significantly reduced, thereafter. This resulted in a severe reduction in maximum ETR (Fig. 2B) and in a reduced photosynthetic efficiency (α). Lower respiratory efficiency (β) was also observed in heat wave exposed cells (Fig. 2C).

Cells grown at 26 °C, for three days, show significantly higher values in the size of the oxidized quinone pool (Area), higher active oxygen evolving complexes (AOEC), and higher energy needed to close all reaction centres than cells grown at 18 °C (S_M) (Fig. 3). The total number of electrons transferred into electron transport chain (N) is higher than the one measured in control cells. No difference was observed in electron transport flux per cross section (ET_O/CS). Heat wave exposed cells also exhibited higher energy absorption (ABS/CS), entrapment (TR_O/CS) and dissipation (DI_O/CS) of energy (Fig. 4). No difference was observed in the reduction rate of the Q_A (M_O), the efficiency with which a PSII trapped electron is transferred from Q_A to Q_B (ΨE_O) and in grouping probability which measures the connectivity between the two PSII units (P_G) (Fig. 5).

Elevated temperature induced changes on the pigment profiles in *P. tricornutum* cells (Fig. 6). Heat wave exposed cells showed significantly higher ($p \leq 0.05$) concentrations of chlorophylls *a* and *c* (Fig. 6A), consequently leading to a higher Chl *a*/*c* ratio (Fig. 6C). Heat wave also induced a significantly higher ($p \leq 0.05$) accumulation of some of the xanthophyll cycle pigments: Ddx, Dtx and Fx (Fig. 6B). Both β-carotene content and DES were not significantly affected by the temperature shift.

Regarding the carbon fixation metabolism and respiration, cells of *P. tricornutum* grown at 18 °C showed significantly higher ($p \leq 0.05$) photosynthetic oxygen production rates than those exposed to 26 °C. On the other hand, the respiratory oxygen consumption rates were not significantly different (Fig. 7). These changes resulted in significantly different ($p \leq 0.05$) respiration/photosynthesis ratios, which was 0.17 ± 0.018 at 18 °C and increased to 0.24 ± 0.049 in heat wave-exposed cells (data not shown).

3.3. Fatty acids and lipid peroxidation products

The fatty acid composition and contents of *P. tricornutum* cells were

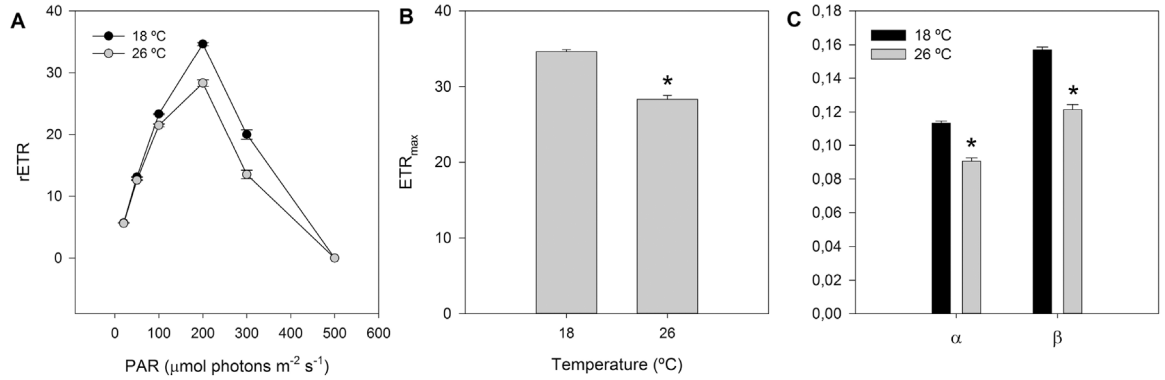

Fig. 2. Rapid light curves (RLC) and derived parameters in P. tricornutum grown at 18 °C and exposed to a heat wave (26 °C, for three days). A) Relative electron transport rate (rETR) at different light intensities; B) Maximum ETR (ETR_{max}); C) Photosynthetic efficiency of PSII (α) and RLC related respiration (β). Values correspond to average ± standard error, n = 3; asterisks indicate significant differences ($p \leq 0.05$).

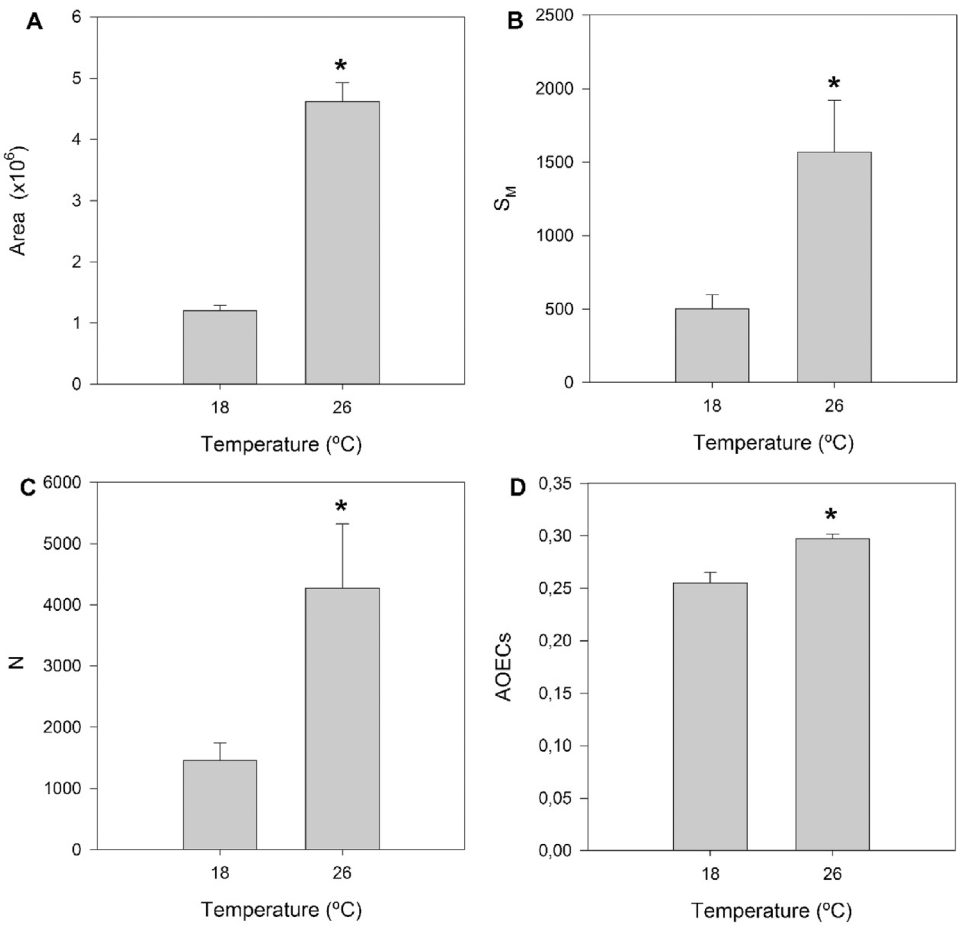

Fig. 3. Parameters derived from OJIP transient curves in P. tricornutum grown at 18 °C and exposed to a heat wave (26 °C, for three days). A) Size of the oxidized quinone pool (Area); B) Energy needed to close all reaction centres (S_M). C) Total number of electrons transferred into electron transport chain (N); D) Active oxygen evolving complexes (AOECs). Values correspond to average ± standard error, n = 3; asterisks indicate significant differences ($p \leq 0.05$).

analyzed by direct trans-esterification of cell pellets. The major fatty acids identified in *P. tricornutum,* under the study culture conditions, were the saturated myristic (C14:0) and palmitic (C16:0) acids, the monounsaturated palmitoleic acid (C16:1), the di-unsaturated hexadecadienoic acid (C16:2n-4), the tri-unsaturated hexadecatrienoic acid (C16:3) and the LC-PUFA EPA (C20:5). Smaller amounts of hexadecatetraenoic acid (C16:4), γ-linolenic acid (C18:3), stearidonic acid (C18:4), arachidonic acid (C20:4), as well as another hexadecadienoic acid (C16:2n-7) were also detected (Fig. 8). Exposure to the heat wave, for three days negatively affected the relative abundance of C20:5, which accounted for ~30% of total FA and dropped to ~20%. A decrease of 11% was also observed for C16:3 and the same trend observed for C16:4. The opposite trend was observed for C16:0, C16:2n-4 and C20:4, which displayed increases of 28, 14 and 56%, respectively, under heat stress. These heat induced changes in the relative proportions of individual FA resulted in a lower DBI and in a higher ω-6/ω-3 ratios (Fig. 9A,B). Regarding the total fatty acids content, on a cell number basis, a significant increase was observed in the heat wave exposed cultures (Fig. 9C).

The level of lipid peroxidation products was assessed by the thiobarbituric acid reactive substances (TBARS) test (Fig. 9D). This value corresponded to 19.5 pmol TBARS per million cells and displayed a reduction in cells exposed to the heat wave (11.8 pmol TBARS per million cells).

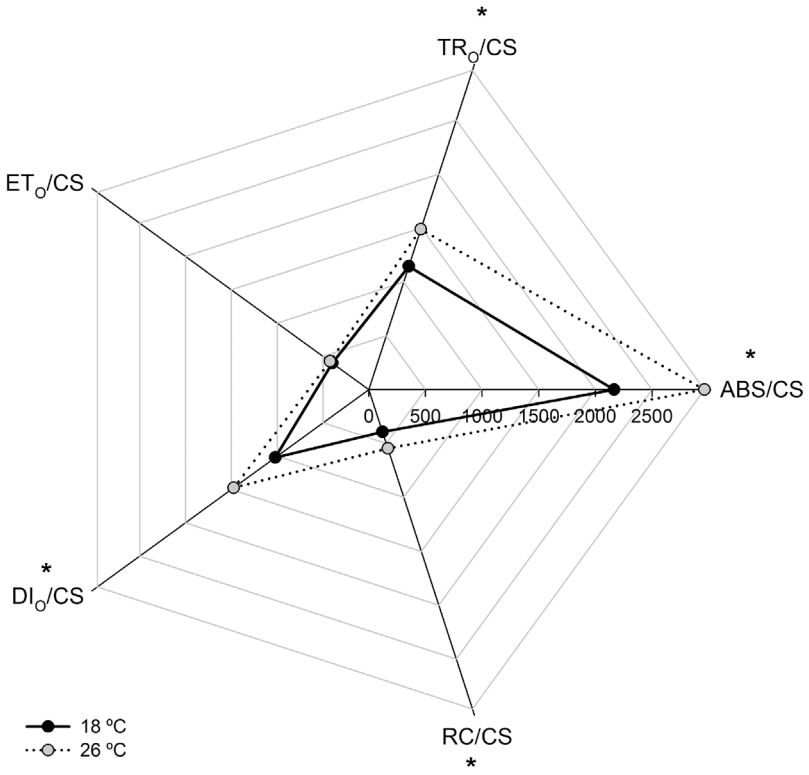

Fig. 4. Phenomenological energy fluxes in P. tricornutum grown at 18 °C (black) and exposed to a heat wave (26 °C, for three days) (gray). Absorbed energy flux per cross-section (ABS/CS); Trapped energy flux per cross-section (TR$_O$/CS); Electron transport energy flux per cross-section (ET$_O$/CS); Dissipated energy flux per cross-section (DI$_O$/CS). Values correspond to average ± standard error, n = 3; asterisks indicate significant differences ($p \leq 0.05$).

4. Discussion

The recent IPCC report on climatic changes (IPCC, 2014) points to extreme thermic events as major threats to marine organisms and ecosystems. Although diatoms have been described by their extraordinary capacity to withstand various types of stress (Falciatore et al., 2000; Cabrita et al., 2016), the fact that they account for approximately 20% of the global primary photosynthetic production, implies that stress-induced changes on diatom growth and physiology will greatly impact higher trophic levels and the ecosystem as a whole. Therefore, the identification, under laboratory controlled conditions, of thermal stress biomarkers that could easily be used to the early detection of heat wave events in marine systems, is of increasing importance.

Diatoms are cosmopolitan species, being the most abundant group in most estuarine systems like the Tagus estuary (Gameiro and Brotas, 2010). This makes these organisms highly suitable as sentinel species for early warning signs, not only for sudden climatic changes such as heat-wave events, but also for chemical stressors.

In the present work we used the same *P. tricornutum* strain previously used by our team to investigate the effects of other types of environmental stressors, such as high light stress (Domingues et al., 2012) and trace element pollution (Cabrita et al., 2016; Matos et al., 2016). Cells were analyzed during the exponential phase in order to minimize the effects of nutrient depletion and culture aging, resulting from long experimental periods (Cabrita et al., 2016). After 3 days of exposure to elevated temperature (26 °C) simulating heat wave conditions, a decrease in *P. tricornutum* cell number, growth rates, and to a larger extent, fresh biomass, was found in comparison to 18 °C grown cells, in accordance with reported optimal and lethal temperatures at 20 °C and between 27 and 30 °C, respectively (Goldman 1977; Sigaud and Aidar, 1993). Accentuated reduction of fresh biomass at 26 °C has been previously observed by Yongmanitchai and Ward (1991) for temperatures higher than 22 °C. since net primary productivity is defined as the difference between photosynthesis and autotrophic respiration, photosynthetic organisms increase their structural biomass and/or their pool of reserves when net productivity has a positive value. The reduction in biomass observed at 26 °C may be linked to the lowered photosynthesis/respiration ratio observed, mainly due to lower

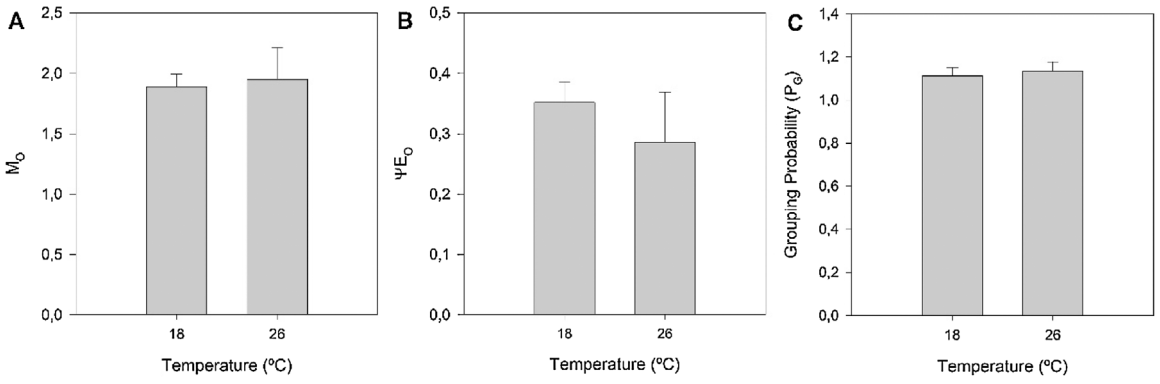

Fig. 5. Observed rate of Quinone A (Q_A) reduction (M_O), efficiency with which a PSII trapped electron is transferred from Q_A to Q_B and grouping probability (P_G) of samples of P. tricornutum at 18 °C and exposed to a heat wave (26 °C, for three days). Values correspond to average ± standard error, n = 3; asterisks indicate significant differences ($p \leq 0.05$).

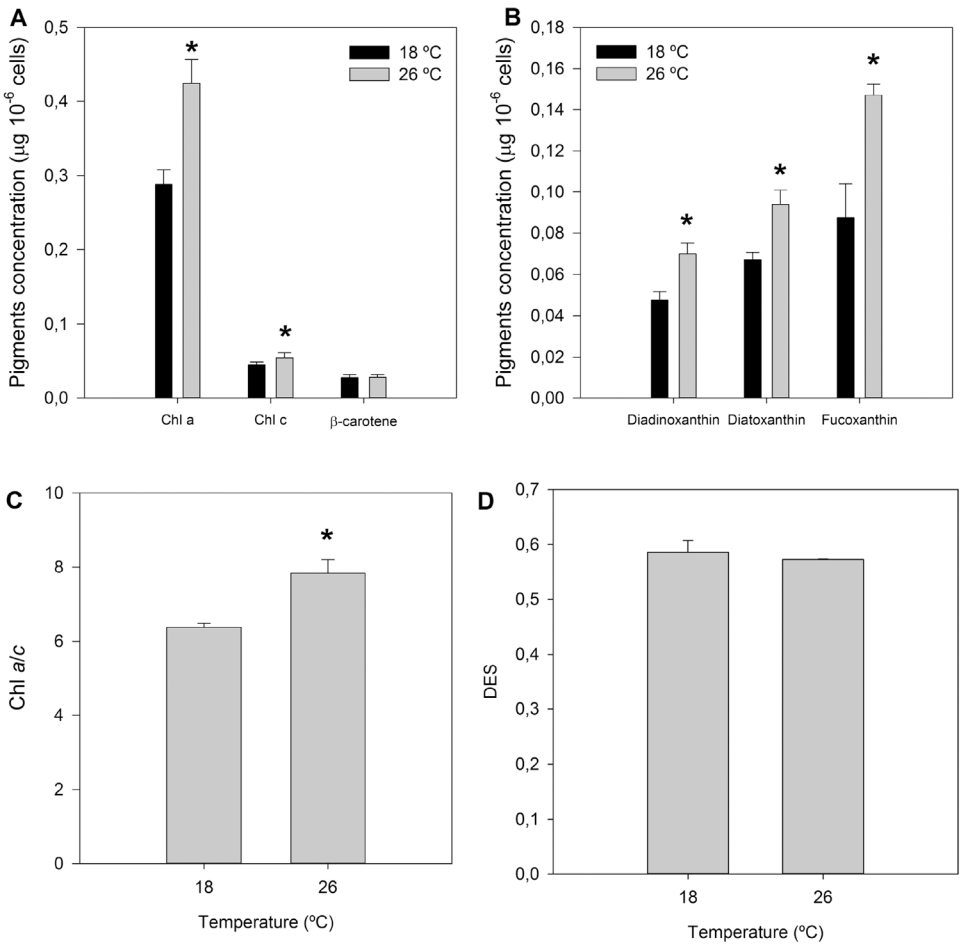

Fig. 6. Pigment composition of P. tricornutum grown at 18 °C (black) and exposed to a heat wave (26 °C, for three days) (gray) A) Chlorophyll a (Chl a), chlorophyll c (Chl c) and β-carotene concentrations; B) Diadinoxanthin (Ddx), Diatoxanthin (Dtx) and Fucoxanthin (Fx) concentrations; C) Ratio of Chlorophyll a and c; D) De-epoxidation state (DES). Values correspond to average ± standard error, n = 3; asterisks indicate significant differences ($p \leq 0.05$).

photosynthetic O_2 production, as the respiratory rate is maintained, indicating that less carbon is used for the production of biomass under heat stress. Differences in water content or silicate morphology, known to be affected by temperature (Javaheri et al., 2015), could also have contributed to the lowered fresh biomass at 26 °C. Furthermore, some reports indicate that under controlled laboratory conditions, the intraspecific cell size of microalgae tends to decrease with increasing temperature (Svensson et al., 2014). Besides the differences associated with cell growth, it is noteworthy that the percentage of clamps was reduced in heat wave exposed cells, associated with an increase of cells grouped in pairs, reinforcing the notion that heat stress impacts cell growth, as reported previously (Ukeles, 1958). Extending the exposure period for a few more days, as applied in other similar studies focusing on effects of environmental stressors in diatom species (Bojko et al., 2013), would have allowed to detect more accentuated differences between the 18 and 26 °C treatments. Nevertheless, in our previous

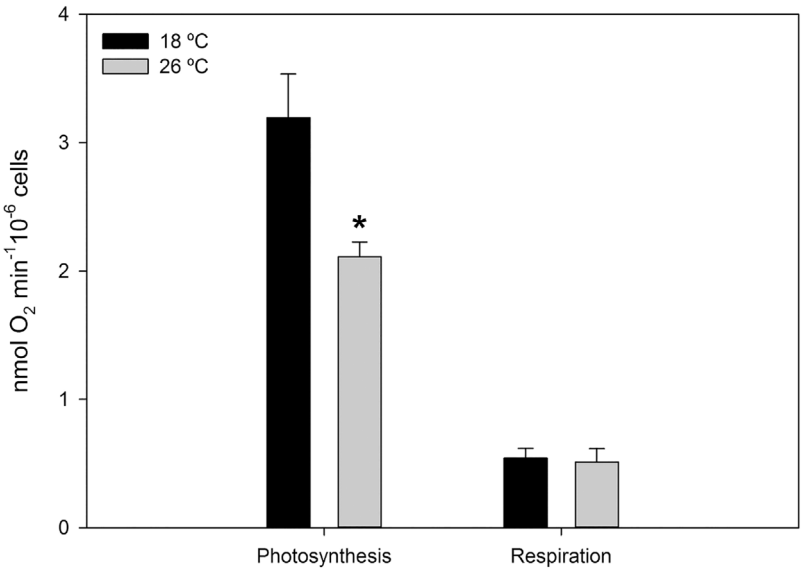

Fig. 7. Photosynthetic and respiratory O_2 flux rates of P. tricornutum at 18 °C (black) and exposed to a heat wave (26 °C, for three days) (gray). Values correspond to average ± standard error, n = 3; asterisks indicate significant differences ($p \leq 0.05$).

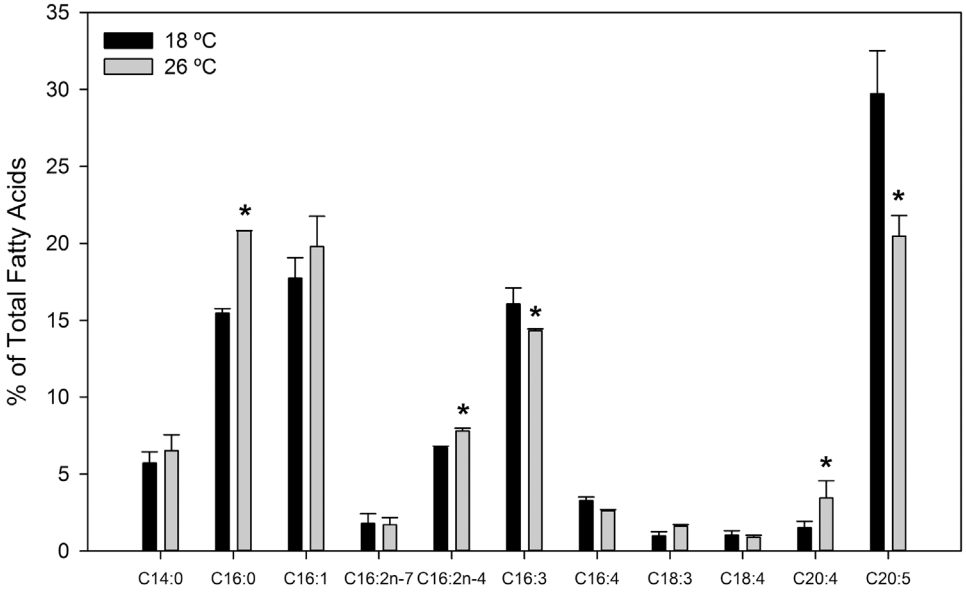

Fig. 8. Fatty acid composition of P. tricornutum grown at 18 °C (black) and exposed to a heat wave (26 °C, for three days) (gray). Values correspond to average ± standard error, n = 3; asterisks indicate significant differences ($p \leq 0.05$).

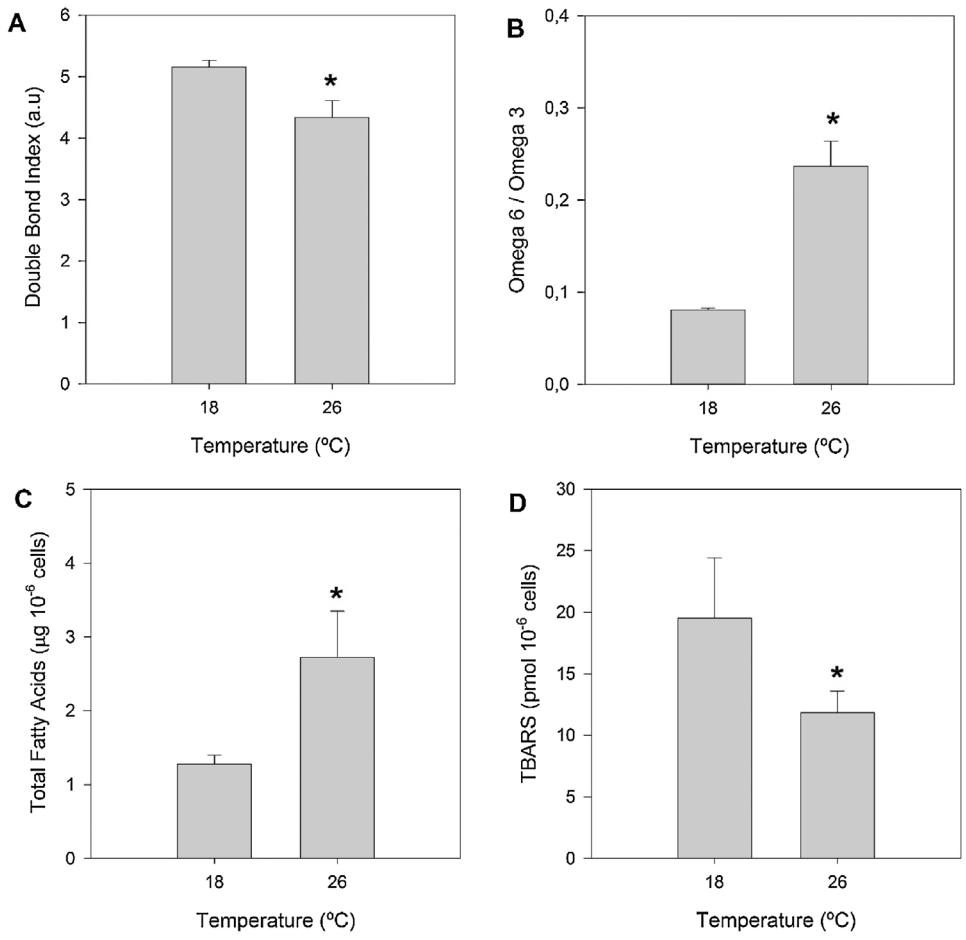

Fig. 9. Lipid-related parameters of P. tricornutum grown at 18 °C and exposed to a heat wave (26 °C, for three days) A) Double bond index (DBI); B) Ratio ω-6/ω-3 fatty acids; C) Total fatty acids content; D) Lipid peroxidation products (expressed as the contents of thiobarbituric acid reacting substances (TBARS)). Values correspond to average ± standard error, n = 3; asterisks indicate significant differences ($p \leq 0.05$).

studies, cell aging has been detected in *P. tricornutum* cultures after 6 days, obscuring the physiological responses to environmental stressors.

The linear relationship between cell concentration and basal fluorescence (F_t), evidencing higher F_t associated with cells exposed to heat wave conditions, suggests the use of this parameter to efficiently evaluate the cell density of *P. tricornutum* cultures. The application of this remote-sensing technique is significantly less time consuming than microscopic cell counting, allowing F_t to be used as an efficient biomarker of biomass changes under stress conditions, like the heat wave stress observed in marine ecosystems.

Photosynthesis is, among cell functions, one of the most sensitive to high temperatures, being the oxygen-evolving complex of photosystem II (PSII) particularly affected by this stress (Glatz et al., 1999). This

complex process includes two different but complementary pathways: light harvesting and carbon fixation. Our combined results of the PAM analysis and the study of oxygen evolution rates indicate that the heat wave exposure negatively impacted photosynthesis whereas mitochondrial respiratory rates, measured at growth temperature, were not affected. Such results reinforce the accepted idea that current heat wave events, as those reported for the Mediterranean area (Gameiro et al., 2007), where water temperature reached the value tested in our study, could negatively impact primary production. Despite the fact that oxygen solubility in water is known to decrease with temperature, under heat stress diatoms will also further contribute less to water oxygenation. Our results indicate that the photosynthesis/respiration ratio, is likely to be a good heat stress indicator, as photosynthesis and respiration are two fundamental and crucial physiological processes, contributing to an integrative understanding of the effects of key abiotic stresses, such as heat (Xu et al., 2015). Nevertheless, the chloroplastidial respiration, essential for energy dissipation under light intensities beyond photo-inhibitory ones, was seen as a relevant mechanism for the cells adaptation to stress allowing an efficient energy dissipation.

Oxygen evolving complexes (OECs) are essential for the water-splitting reaction occurring at the donor side of the PSII and essential not only for oxygen production but also for fueling the quinone pool with the necessary energy for electron transport from PSII to PSI (Strasser et al., 2000). These complexes are usually heat-sensitive (Duarte et al., 2016). Nevertheless, in the present study *P. tricornutum*, OECs were shown to be highly thermo-tolerant as observed by the increase in the percentage of activated OECs and number of electrons transferred into the electron transport chain in heat wave exposed cells. This contrasts with the marine plant *Spartina maritima,* an halophyte, in which OECs had low thermo-stability under heat stress (Duarte et al., 2016). However, this high activity at the PSII donor side and the unchanged size of the oxidized quinone pool available to receive the generated electrons, showed that the electron transport was severely impaired, indicating problems either in the PSII intersystem or in the functioning of the quinone pool (Duarte et al., 2016). This leads to the accumulation of excessive reducing power inside the chloroplast that needs to be dissipated to prevent the PSII destruction due to D1 protein unfolding (Duarte et al., 2016; Domingues et al., 2012). One of the main physiological processes involved in the thermal dissipation of harmful excess energy is the xanthophyll cycle. In diatoms this cycle, involves the deepoxidation of the pigment Ddx to Dtx, leading to non-photochemical quenching (NPQ) in the antenna pigment-protein complexes decreasing the excitation rate of PSII reaction centres. Nevertheless, this was not observed in the present work, pointing to other energy dissipation mechanisms. In fact, this enzymatic pathway is not the only energy dissipation pathway contributing to non-photochemical quenching. In the present study, the energy dissipation in the form of heat throughout energetic fallouts of the excited chlorophyll molecules seemed to acquire the most relevant role, as observed by an increased DI/RC. This was previously verified for other marine photosynthetic organisms (Duarte et al., 2016). In the past, Ruban et al. (2004) showed that *P. tricornutum* could form NPQ 3 to 5 times larger than higher plants, which may be a central feature explaining the success of diatoms in variable light environments (Lavaud et al., 2004).

Heat-wave exposed samples had higher concentrations of Chl *a* and Fx which is the only carotenoid with an efficient light harvesting function (Mann and Myers, 1968), being a component of the fucoxanthin-chlorophyll protein (FCP) complex. Additionally, Fx is also able to act as an antioxidant (Foo et al., 2017; Zhao et al., 2014) since it can compete with oxygen for electrons. This is intrinsically connected with the higher number of active reaction PSII centres in heat-wave exposed *P. tricornutum* cells, due to an increase in the number of FC complexes and absorption of energy (ABS/CS). This is reinforced with a higher energy needed for the closing of all the reaction centres. Significant changes could be observed in the size of the quinone pool between the 18- and 26 °C exposed cells, this showing a strong positive correlation with the concentration of Chl *a* and Fx ($\rho = 0.886$ and $\rho = 0.829$, respectively) and indicating a higher need of transport of more harvested energy. Nevertheless, and as abovementioned, this was not followed by an increase in the electron transport rate from Q_A a to Q_B (ET_O/CS) in heat exposed cells, so the excessive energy that is not able to be quickly transported had to be dissipated as heat, decreasing the efficiency of the photosystem II and activating the photoprotective mechanisms as previously reported for higher plants (Duarte et al., 2015). Exposure to 26 °C did not influence the reduction rate of the Q_A (M_O) ($p = 0.513$), or the efficiency with which an absorbed photon results in electron transport beyond Q_{A-} (ΨE_O) ($p = 0.513$) which explains why the electron transport flux did not increase, suggesting that the size of the pool of oxidized quinones increased to face the sudden increase of electrons being transferred into the electron transport chain which also showed a strong positive correlation ($\rho = 1.000$).

Our results indicate a strong negative correlation between photosynthetic pigments like Chl *a* and Fx and the lower photosynthetic efficiency (α) ($\rho = -0.886$ and $\rho = -0.829$, respectively), suggesting that a decrease in the photosystem II efficiency could induce an increase in the content of light harvesting pigments, as a feedback mechanism counteracting the energy deficit. Higher Chl *a* contents were previously reported for *P. tricornutum* grown at 25 °C, in comparison with cells grown at 10 °C (Thompson et al., 1992), in agreement with the similar results obtained by Bojko et al. (2013) and (Hemalatha et al., 2012) for the marine diatom *Chaetoceros simplex*, suggesting a common adaptation mechanism in this family. The opposite trend was observed when culture temperature decreased from 20 to 10 °C (Kudo et al., 2000).

Although there were no signs of the involvement of the xanthophyll cycle in the energy dissipation of heat exposed cells, significant changes could be observed in Dtx contents, suggesting another important role of this carotenoid. Lavaud et al. (2004) found that a correlation existed between Dtx and NPQ and hypothesised that Dtx could play a role in preventing lipid peroxidation. Kuczynska et al. (2015) reported that lipid-bound diadinoxanthin-cycle pigments could play an antioxidant function scavenging singlet oxygen and peroxydized lipids. This might explain, at least in part, the decrease in the concentration of lipid peroxidation products in samples exposed to 26 °C, as both parameters display a strong negative correlation ($\rho = -1.000$). On the other hand, heat wave exposed cells also have a lower DBI and this could also be related to the lower content of lipid peroxidation products ($\rho = 0.829$). Although the optimal growth temperature for *P. tricornutum* is 20 °C and temperatures around 30 °C are lethal (Goldman 1977; Sigaud and Aidar, 1993), it appears that this diatom is able to activate protective mechanisms, which likely contribute to its cosmopolitan behavior. Strikingly, no differences were observed in the β-carotene levels between cells grown at 18 and exposed to 26 °C as reported for abiotic stress conditions, such as high light stress (Haubner et al., 2014). The hypothesis of a higher biosynthetic rate of this antioxidant pigment cannot be excluded, since it might be rapidly consumed, in order to generate increased levels of Fx and Dtx through the diatoxanthin cycle (Mulders et al., 2014), as previously observed in several species of diatoms under stress (Xia et al., 2013; Masmoudi et al., 2013), including *P. tricornutum* (Grouneva et al., 2009; Oilaizola et al., 1995). The set of photochemical parameters with important physiological meaning used in this study can be a useful battery of biomarkers for future heat wave risk assessment studies.

Lipids play key roles in the regulation of membrane fluidity under temperature stress (Los and Murata, 2004; Horváth et al., 2012; Matos et al., 2007). Besides being membrane components, fatty acids are also present in storage lipids and play fundamental roles in cell signaling pathways. Oleaginous microalgae, including diatoms, are considered as a promising resource for the production of biofuels, since they are the most productive in carbon fixation and very flexible to environmental changes (Popko et al., 2016). The lack of nutrients and temperature stress are commonly used to induce storage lipid accumulation in *P.*

tricornutum and other microalgae, however, these stresses inevitably inhibit growth (Du and Benning 2016). The effect of temperature on lipid accumulation in several microalgae has been reviewed by Sharma et al. (2012). As a general trend, a decrease in the unsaturation level of membrane lipids is observed, leading to a decrease in the DBI, whereas an increase in the total lipid content generally occurs at higher temperatures. Although the total fatty acid profiles of *P. tricornutum* cells consist of a mixture of the acyl chains present in membrane- and storage lipids, the particular lipid metabolism of *P. tricornutum*, characterized by a high variety of fatty acids and with distinct signatures for different lipid classes (Abida et al., 2015; Popko et al., 2016), allows to draw significant information related to key plastidial lipids. Moreover, such results can be obtained, by direct trans-esterification of cells, coupled to GC analysis, skipping the time-consuming lipid extraction step, which is often incomplete (Griffiths et al., 2010).

De novo fatty acid biosynthesis in *P. tricornutum* follows two main pathways (reviewed by Dolch and Maréchal, 2015). The prokaryotic pathway takes place inside the chloroplast, producing saturated fatty acids with 14, 16, and 18 carbons. However, mainly C16:0 is desaturated, by the soluble plastidial desaturase, and C16:1, after being esterified to plastidial membrane glycerolipids, is further desaturated by membrane-bound desaturases, yielding C16:2, C16:3 and C16:4. Fatty acids with more than 18 carbons, including EPA, are synthetized trough the eukaryotic pathway in the endoplasmatic reticulum, from chloroplasts precursors, by the combined activities of elongases and desaturases. Although the complete pathway(s) of EPA synthesis in *P. tricornutum* is not fully elucidated, it likely involves elongation of 20:4 (Dolch and Maréchal, 2015). EPA is further released from phospholipids, into the cytosolic acyl-CoA pool, and incorporated in plastidial lipids, by a yet unknown mechanism, designated Omega pathway (Petroutsos et al., 2014), resulting in the presence of EPA in all membrane lipids. In contrast, significant amounts of C16:3, and, to a lower extent, C16:4 are found only in plastidial lipids, mainly in monogalactosildiacylglycerol (MGDG), which forms the bulk of thylakoid lipids. Results obtained in the present study, indicating a lower DBI and a higher total fatty acid content at 26 °C are also in agreement with previous reports on *P. tricornutum* (Yongmanitchai and Ward 1991; Dodson et al., 2014). The lower amount of EPA and higher amounts of the saturated C16:0, found here in heat wave exposed cells, likely reflect the negative impact of high temperature on the fatty acid desaturation mechanisms. Moreover, regarding the prokaryotic pathway, the lower amount of C16:3 and the same tendency observed for C16:4 in heat treated samples, suggests a decrease in MGDG contents, which is likely related to the negative effects of heat at the photosynthesis level. In contrast, the higher proportion of C16:2, present in the plastidial digalactosildiacylglycerol (DGDG) and the extraplastidial phosphatidylcholine, might indicate an increase of these lipid classes. Although an increase in the ratio DGDG/MGDG has been suggested as a mechanism to cope with stress in higher plants (Gigon et al., 2004), to our knowledge, no data concerning this ratio is reported for *P. tricornutum*, although it was observed that this ratio is altered in response to light in Antarctic sea diatoms (Mock and Kroon, 2002). The differences on total fatty acid profiles observed between cells cultured at 18 and 26 °C, likely result from changes in the acyl composition of individual lipids, but they can also reflect alterations in the relative amounts of each lipid class. For instance, storage lipids such as triacylglycerols, known to be enriched in saturated fatty acids (Abida et al., 2016) are known to accumulate under stress conditions (Sharma et al., 2012). The effect of the heat wave on the relative amounts of each lipid classes as well as their individual fatty acid composition in *P. tricornutum* remains to be investigated and shall be the focus of our future work.

In sum, several fatty-acid related biomarkers can be proposed to assess the impact of heat wave events on *P. tricornutum*. The relative content of the saturated, C16:0, versus the LC-PUFA, EPA, as well as the ω-6/ω-3 ratio, seem to be good indicators of stress, as both increased under the heat wave. Similar conclusions can be provided by the DBI, which is negatively related to the temperature increase. Moreover, the percentage of C16:3, and eventually C16:4, appear as good indicators of thylakoid lipids and possible consequences of these temperature-related changes on photosynthesis.

Regarding potential effects to the food web, from our results and previous data, it is logical that climate changes leading to the occurrence of heat wave events are likely to result in lowering the contents of the ω-3 LC-PUFA, such as EPA, in phytoplankton. Because EPA is transferred to higher trophic levels of marine food webs, where most organisms have limited ability to produce it, changes in the fatty acid composition of phytoplankton will cause alterations in the structure of food chains, and consequently affect marine organisms and communities (Vagner et al., 2015). Moreover, increased respiration/photosynthesis ratios will lead not only to lower net primary productivity but also contribute to water deoxygenation, with impacts to the marine ecosystem as a whole.

5. Conclusions

Altogether, our results showed that the model diatom *P. tricornutum* provides several biomarkers able to efficient assess the harmful effects of a heat wave event. The non-invasive JIP-test produces a wide number of parameters, including electron transport related-features that, beyond its undeniable physiological importance, accurately responded to the heat stress to which the cells were subjected. Other biomarkers obtained by biochemical analysis, including spectrophotometric pigment profiling of fatty acid analysis by direct-transesterification coupled to GC, allowed the identification of the saturated FA, C16:0, versus the LC-PUFA, EPA, as well as the ω-6/ω-3 ratio as good stress indicators. Although in the present work the effects of a heat wave were analyzed under controlled laboratory conditions, in a model species, the proposed battery of biomarkers can also be applied to field assessment of ecological impacts of heat stress.

Acknowledgements

The authors would like to thank the “Fundação para a Ciência e Tecnologia (FCT)” for funding the research in the Marine and Environmental Sciences Centre (MARE) throughout the project UID/MAR/04292/2013 and the Biosystems and Integrative Sciences Institute (BioISI) throughout the project UID/MULTI/04046/2013. This specific work was preformed within the framework of the Hg-Planktartic project, part of the Portuguese Polar Program (PROPOLAR, FCT funded). B. Duarte and M.T. Cabrita investigation was supported by FCT throughout Post-doctoral grants (SFRH/BPD/115162/2016 and SFRH/BPD/50348/2009, respectively). The authors wish to thank Rui Malhó support to microscopy work, João D. Arrabaça and Miguel Gaspar for their help with the oxygen electrode and Manuela Lucas for technical assistance.

References

Abida, H., Dolch, L.-J., Meï, C., Villanova, V., Conte, M., Block, M.A., Finazzi, G., Bastien, O., Tirichine, L., Bowler, C., Rébeillé, F., Petroutsos, D., Jouhet, J., Maréchal, E., 2015. Membrane glycerolipid remodeling triggered by nitrogen and phosphorus starvation in *Phaeodactylum tricornutum*. Plant Physiol. 167, 118–136. http://dx.doi.org/10.1104/pp.114.252395.

Anjum, N.A., Duarte, B., Caçador, I., Sleimi, N., Duarte, A.C., Pereira, E., 2016. Biophysical and biochemical markers of metal/metalloid-impacts in salt marsh halophytes and their implications. Front. Environ. Sci. 4, 1–13. http://dx.doi.org/10.3389/fenvs.2016.00024.

Arts, M.T., Ackman, R.G., Holub, B.J., 2001. Essential fatty acids in aquatic ecosystems: a crucial link between diet and human health and evolution. Can. J. Fish. Aquat. Sci. 58, 122–137. http://dx.doi.org/10.1139/f00-224.

Baldi, M., Pasqui, M., Cesarone, F., De Chiara, G., 2005. Heat waves in the mediterranean region: analysis and model results. 16th Conf. Clim. Var. Change.

Bojko, M., Brzostowska, K., Kuczynska, P., Latowski, D., Olchawa-Pajor, M., Krzeszowiec, W., Waloszek, A., Strzalka, K., 2013. Temperature effect on growth, and selected parameters of *Phaeodactylum tricornutum* in batch cultures. Acta Biochim. Pol. 60, 861–864 PMID:24432347.

Budge, S.M., Devred, E., Foret, M.-H., Stuart, V., Trzcinski, M.K., Sathyendranath, S., Platt, T., 2014. Estimating concentrations of essential omega -3 fatty acids in the ocea:supply and demand. ICES J. Mar. Sci. http://dx.doi.org/10.1093/icesjms/fsu003.

Cabrita, M.T., Gameiro, C., Utkin, A.B., Duarte, B., Caçador, I., Cartaxana, P., 2016. Photosynthetic pigment laser-induced fluorescence indicators for the detection of changes associated with trace element stress in the diatom model species *Phaeodactylum tricornutum*. Environ. Monit. Assess. 188. http://dx.doi.org/10.1007/s10661-016-5293-4.

Dalsgaard, J., St. John, M., Katnner, G., Muller-Navarra, D.C., Hagen, W., 2003. Fatty acid trophic markers in the pelagic marine food environment. Adv. Mar. Biol. 2003 (46), 225–340 PMID: 14601414.

Dasari, H.P., Pozo, I., Ferri-Yáñez, F., Araújo, M.B., 2014. A regional climate study of heat waves over the Iberian Peninsula. Atmos. Clim. Sci. 4, 841–853. http://dx.doi.org/10.4236/acs.2014.45074.

De Martino, A., Bartual, A., Willis, A., Meichenin, A., Villazán, B., Maheswari, U., Bowler, C., 2011. Physiological and molecular evidence that environmental changes elicit morphological interconversion in the model diatom *Phaeodactylum tricornutum*. Protist 162, 462–481. http://dx.doi.org/10.1016/j.protis.2011.02.002.

Dodson, V.J., Mouget, J.L., Dahmen, J.L., Leblond, J.D., 2014. The long and short of it: temperature-dependent modifications of fatty acid chain length and unsaturation in the galactolipid profiles of the diatoms Haslea ostrearia and *Phaeodactylum tricornutum*. Hydrobiologia 727, 95–107. http://dx.doi.org/10.1007/s10750-013-1790-4.

Dolch, L.J., Maréchal, E., 2015. Inventory of fatty acid desaturases in the pennate diatom *Phaeodactylum tricornutum*. Mar. Drugs 13, 1317–1339. http://dx.doi.org/10.3390/md13031317.

Domingues, N., Matos, A.R., da Silva, J.M., Cartaxana, P., 2012. Response of the diatom *Phaeodactylum tricornutum* to photooxidative stress resulting from high light exposure. PLoS One 7, 1–6. http://dx.doi.org/10.1371/journal.pone.0038162.

Doney, S., Ruckelshaus, M., Duffy, J., Barry, J., Chan, F., English, C., Galindo, H., Grebmeier, J., Hollowed, A., Knowlton, N., Polovina, J., Rabalais, N., Sydeman, W., Talley, L., 2012. Climate change impacts on marine ecosystems. Ann. Rev. Mar. Sci. http://dx.doi.org/10.1146/annurev-marine-041911-111611.

Du, Z.-Y., Benning, C., 2016. Triacylglycerol accumulation in photosynthetic cells in plants and alga. In: Nakamura, Y., Li-Beisson, Y. (Eds.), Lipids in Plant and Algae Development. Springer International Publishing, Cham, pp. 179–205. http://dx.doi.org/10.1007/978-3-319-25979-6_8.

Duarte, B., Goessling, J.W., Marques, J.C., Caçador, I., 2015. Ecophysiological constraints of *Aster tripolium* under extreme thermal events impacts: merging biophysical, biochemical and genetic insights. Plant Physiol. Biochem. 97, 217–228. http://dx.doi.org/10.1016/j.plaphy.2015.10.015.

Duarte, B., Marques, J.C., Caçador, I., 2016. Ecophysiological response of native and invasive Spartina species to extreme temperature events in Mediterranean marshes. Biol. Invasions 18, 2189–2205. http://dx.doi.org/10.1007/s10530-015-0958-4.

Duarte, B., Cabrita, M.T., Gameiro, C., Matos, A.R., Godinho, R., Marques, J.C., Caçador, I., 2017. Disentangling the photochemical salinity tolerance in *Aster tripolium* L.: connecting biophysical traits with changes in fatty acid composition. Plant Biol. 19. http://dx.doi.org/10.1111/plb.12517.

EEA, 2012. Climate Change, Impacts and Vulnerability in Europe 2012, An Indicator Based Report. EEA Report No.12/2012. European Environment Agency (EEA), Copenhagen, Denmark 304 pp.

Falciatore, A., d'Alcalà, M.R., Croot, P., Bowler, C., 2000. Perception of environmental signals by a marine diatom. Science 288, 2363. http://dx.doi.org/10.1126/science.288.5475.2363. LP-2366.

Falk, S., Maxwell, D.P., Laudenbach, D.E., Huner, N.P.A., 1996. Photosynthetic adjustment to temperature. In: Baker, N.R. (Ed.), Photosynthesis and the Environment. Springer, Netherlands, Dordrecht, pp. 367–385. http://dx.doi.org/10.1007/0-306-48135-9_15.

Filimonova, V., Gonçalves, F., Marques, J.C., De Troch, M., Gonçalves, A.M.M., 2016. Fatty acid profiling as bioindicator of chemical stress in marine organisms: a review. Ecol. Indic. 67, 657–672. http://dx.doi.org/10.1016/j.ecolind.2016.03.044.

Foo, S.C., Yusoff, F.M., Ismail, M., Basri, M., Yau, S.K., Khong, N.M.H., Chan, K.W., Ebrahimi, M., 2017. Antioxidant capacities of fucoxanthin-producing algae as influenced by their carotenoid and phenolic contents. J. Biotechnol. 241, 175–183. http://dx.doi.org/10.1016/j.jbiotec.2016.11.026.

Galloway, A.W.E., Winder, M., 2015. Partitioning the relative importance of phylogeny and environmental conditions on phytoplankton fatty acids. PLoS One 10, 1–23. http://dx.doi.org/10.1371/journal.pone.0130053.

Gameiro, C., Brotas, V., 2010. Patterns of Phytoplankton variability in the Tagus estuary (Portugal). Estuar. Coasts 33, 311–323. http://dx.doi.org/10.1007/s12237-009-9194-4.

Gameiro, C., Cartaxana, P., Brotas, V., 2007. Environmental drivers of phytoplankton distribution and composition in Tagus Estuary, Portugal. Estuar. Coast. Shelf Sci. 75, 21–34. http://dx.doi.org/10.1016/j.ecss.2007.05.014.

Gameiro, C., Utkin, A.B., Cartaxana, P., da Silva, J.M., Matos, A.R., 2016. The use of laser induced chlorophyll fluorescence (LIF) as a fast and non-destructive method to investigate water deficit in Arabidopsis. Agric. Water Manag. 164, 127–136. http://dx.doi.org/10.1016/j.agwat.2015.09.008.

Gigon, A., Matos, A.R., Laffray, D., Zuily-Fodil, Y., Pham-Thi, A.T., 2004. Effect of drought stress on lipid metabolism in the leaves of *Arabidopsis thaliana* (Ecotype Columbia). Ann. Bot. 94, 345–351. http://dx.doi.org/10.1093/aob/mch150.

Glatz, A., Vass, I., Los, D. a., Vígh, L., 1999. The Synechocystis model of stress: from molecular chaperones to membranes. Plant Physiol. Biochem. 37, 1–12. http://dx.doi.org/10.1016/S0981-9428(99)80061-8.

Goldman, J.C., 1977. Temperature effects on phytoplankton growth in continuous culture. Limnol. Oceanogr. 22, 932–936. http://dx.doi.org/10.4319/lo.1977.22.5.0932.

González-Dávila, M., 1995. The role of phytoplankton cells on the control of heavy metal concentration in seawater. Mar. Chem. 48, 215–236. http://dx.doi.org/10.1016/0304-4203(94)00045-F.

Griffiths, M.J., Van Hille, R.P., Harrison, S.T.L., 2010. Selection of direct transesterification as the preferred method for assay of fatty acid content of microalgae. Lipids 45, 1053–1060. http://dx.doi.org/10.1007/s11745-010-3468-2.

Grouneva, I., Jakob, T., Wilhelm, C., Goss, R., 2009. The regulation of xanthophyll cycle activity and of non-photochemical fluorescence quenching by two alternative electron flows in the diatoms *Phaeodactylum tricornutum* and *Cyclotella meneghiniana*. Biochim. Biophys. Acta – Bioenergy 1787, 929–938. http://dx.doi.org/10.1016/j.bbabio.2009.02.004.

Guillard, R.R.L., Ryther, J.H., 1962. Studies of marine planktonic diatoms: I. *Cyclotella nana* Hustedt, and Detonula confervacea (Cleve) Gran. Can. J. Microbiol. 8, 229–239. http://dx.doi.org/10.1139/m62-029.

Guschina, I.A., Harwood, J.L., 2009. Algal lipids and effect of the environment on their biochemistry. In: Kainz, M., Brett, M.T., Arts, M.T. (Eds.), Lipids in Aquatic Ecosystems. Springer, New York, NY, pp. 1–24. http://dx.doi.org/10.1007/978-0-387-89366-2_1.

Harley, C.D.G., Hughes, A.R., Hultgren, K.M., Miner, B.G., Sorte, C.J.B., Thornber, C.S., Rodriguez, L.F., Tomanek, L., Williams, S.L., 2006. The impacts of climate change in coastal marine systems. Ecol. Lett. 9, 228–241. http://dx.doi.org/10.1111/j.1461-0248.2005.00871.x.

Haubner, N., Sylvander, P., Vuori, K., Snoeijs, P., 2014. Abiotic stress modifies the synthesis of alpha-tocopherol and beta-carotene in phytoplankton species. J. Phycol. 50, 753–759. http://dx.doi.org/10.1111/jpy.12198.

Heath, R.L., Packer, L., 1968. Photoperoxidation in isolated chloroplasts. Arch. Biochem. Biophys. 125, 189–198. http://dx.doi.org/10.1016/0003-9861(68)90654-1.

Hemalatha, A., Karthikeyan, P., Manimaran, K., Anantharaman, P., Sampathkumar, P., 2012. Effect of Temperature on the growth of marine diatom, chaetoceros simplex (Ostenfeld, 1901) with different nitrate: silicate concentrations. Asian Pac. J. Trop. Biomed. 2, S1817–S1821. http://dx.doi.org/10.1016/s2221-1691(12)60501-2.

Horváth, I., Glatz, A., Nakamoto, H., Mishkind, M.L., Munnik, T., Saidi, Y., Goloubinoff, P., Harwood, J.L., Vigh, L., 2012. Heat shock response in photosynthetic organisms: membrane and lipid connections. Prog. Lipid Res. 51, 208–220. http://dx.doi.org/10.1016/j.plipres.2012.02.002.

IPCC, et al., 2014. Climate Change 2014: Impacts, Adaptation and Vulnerability. Part A. Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. In: Field, C.B. (Ed.), Cambridge University Press, Cambridge, UK.

Javaheri, N., Dries, R., Burson, a., Stal, L.J., Sloot, P.M. a., Kaandorp, J. a., 2015. Temperature affects the silicate morphology in a diatom. Sci. Rep. 5, 11652. http://dx.doi.org/10.1038/srep11652.

Küpper, H., Seibert, S., Parameswaran, A., 2007. Fast, sensitive, and inexpensive alternative to analytical pigment HPLC: quantification of chlorophylls and carotenoids in crude extracts by fitting with Gauss peak spectra. Anal. Chem. 79, 7611–7627. http://dx.doi.org/10.1021/ac070236m.

Kuczynska, P., Jemiola-Rzeminska, M., Strzalka, K., 2015. Photosynthetic pigments in diatoms. Mar. Drugs 13, 5847–5881. http://dx.doi.org/10.3390/md13095847.

Kudo, I., Miyamoto, M., Noiri, Y., Maita, Y., 2000. Combined effects of temperature and iron on the growth and physiology of the marine diatom *Phaeodactylum tricornutum* (Bacillariophyceae). J. Phycol. 36, 1096–1102. http://dx.doi.org/10.1046/j.1529-8817.2000.99042.x.

Lavaud, J., Rousseau, B., Etienne, A.-L., 2004. General features of photoprotection by energy dissipation in planktonic diatoms (Bacillariophyceae)1. J. Phycol. 40, 130–137. http://dx.doi.org/10.1046/j.1529-8817.2004.03026.x.

Los, D.A., Murata, N., 2004. Membrane fluidity and its roles in the perception of environmental signals. Biochim. Biophys. Acta – Biomembr. 1666, 142–157. http://dx.doi.org/10.1016/j.bbamem.2004.08.002.

Mühlroth, A., Li, K., Røkke, G., Winge, P., Olsen, Y., Hohmann-Marriott, M.F., Vadstein, O., Bones, A.M., 2013. Pathways of lipid metabolism in marine algae, co-expression network, bottlenecks and candidate genes for enhanced production of EPA and DHA in species of chromista. Mar. Drugs 11, 4662–4697. http://dx.doi.org/10.3390/md11114662.

Mann, J.E., Myers, J., 1968. Photosynthetic enhancement in the diatom *Phaeodactylum tricornutum*. Plant Physiol. 43, 1991–1995. http://dx.doi.org/10.1104/pp.43.12.1991.

Masmoudi, S., Nguyen-Deroche, N., Caruso, A., Ayadi, H., Morant-Manceau, A., Tremblin, G., Bertrand, Martine, Schoefs, B., 2013. Cadmium, copper, sodium and zinc effects on diatoms: from heaven to hell – a review. Cryptogam. Algol. 34, 185–225. http://dx.doi.org/10.7872/crya.v34.iss2.2013.185.

Matos, A.R., Hourton-Cabassa, C., Ciçek, D., Rezé, N., Arrabaça, J.D., Zachowski, A., Moreau, F., 2007. Alternative oxidase involvement in cold stress response of *Arabidopsis thaliana* fad2 and FAD3+ cell suspensions altered in membrane lipid composition. Plant Cell Physiol. 48, 856–865. http://dx.doi.org/10.1093/pcp/pcm061.

Matos, A.R., Mendes, A.T., Scotti-Campos, P., Arrabaça, J.D., 2009. Study of the effects of salicylic acid on soybean mitochondrial lipids and respiratory properties using the alternative oxidase as a stress-reporter protein. Physiol. Plant. 137, 485–497. http://dx.doi.org/10.1111/j.1399-3054.2009.01250.x.

Matos, A.R., Gameiro, C.L., Duarte, B., Caçador, I., Cabrita, M.T., 2016. Effects of nickel on the fatty acid composition of the diatom *Phaeodactylum tricornutum*. Front. Mar. Sci. http://dx.doi.org/10.3389/conf.fmars.2016.04.00033.

Mock, T., Kroon, B.M.A., 2002. Photosynthetic energy conversion under extreme conditions – I: important role of lipids as structural modulators and energy sink under N-

limited growth in Antarctic sea ice diatoms. Phytochemistry 61, 41–51. http://dx.doi.org/10.1016/S0031-9422(02)00216-9.

Mulders, K.J.M., Lamers, P.P., Martens, D.E., Wijffels, R.H., 2014. Phototrophic pigment production with microalgae: biological constraints and opportunities. J. Phycol. 50, 229–242. http://dx.doi.org/10.1111/jpy.12173.

Niu, S., Luo, Y., Li, D., Cao, S., Xia, J., Li, J., Smith, M.D., 2014. Plant growth and mortality under climatic extremes: an overview. Environ. Exp. Bot. 98, 13–19. http://dx.doi.org/10.1016/j.envexpbot.2013.10.004.

OECD, Organisation for Economic Co-operation and Development, Freshwater algal and cyanobacteria, growth inhibition test – test guideline 201, 2002. OECD Guidelines for the testing of chemicals, Paris, France, vol. 1 (2), pp. 1–25.

Oilaizola, M., La Roche, J., Kolber, Z., Falkowski, P.G., 1995. Non-photochemical fluorescence quenching in the diadinoxanthin cycle in a marine diatom. Photosynth. Res. 41, 357–370.

Parrish, C.C., 2009. Essential fatty acids in aquatic food webs. In: Kainz, M., Brett, M.T., Arts, M.T. (Eds.), Lipids in Aquatic Ecosystems. Springer, New York, NY, pp. 309–326. http://dx.doi.org/10.1007/978-0-387-89366-2_13.

Petroutsos, D., Amiar, S., Abida, H., Dolch, L.J., Bastien, O., Rébeillé, F., Jouhet, J., Falconet, D., Block, M.A., McFadden, G.I., Bowler, C., Botté, C., Maréchal, E., 2014. Evolution of galactoglycerolipid biosynthetic pathways – from cyanobacteria to primary plastids and from primary to secondary plastids. Prog. Lipid Res. 54, 68–85. http://dx.doi.org/10.1016/j.plipres.2014.02.001.

Popko, J., Herrfurth, C., Feussner, K., Ischebeck, T., Iven, T., Haslam, R., Hamilton, M., Sayanova, O., Napier, J., Khozin-Goldberg, I., Feussner, I., 2016. Metabolome analysis reveals betaine lipids as major source for triglyceride formation, and the accumulation of sedoheptulose during nitrogen-starvation of *Phaeodactylum tricornutum*. PLoS One 11, 1–23. http://dx.doi.org/10.1371/journal.pone.0164673.

Ruban, A., Lavaud, J., Rousseau, B., Guglielmi, G., Horton, P., Etienne, A.-L., 2004. The super-excess energy dissipation in diatom algae: comparative analysis with higher plants. Photosynth. Res. 82, 165. http://dx.doi.org/10.1007/s11120-004-1456-1.

Santos-Ballardo, D.U., Rossi, S., Hernández, V., Gómez, R.V., del Carmen Rendón-Unceta, M., Caro-Corrales, J., Valdez-Ortiz, A., 2015. A simple spectrophotometric method for biomass measurement of important microalgae species in aquaculture. Aquaculture 448, 87–92. http://dx.doi.org/10.1016/j.aquaculture.2015.05.044.

Sharma, K.K., Schuhmann, H., Schenk, P.M., 2012. High lipid induction in microalgae for biodiesel production. Energies 5, 1532–1553. http://dx.doi.org/10.3390/en5051532.

Sigaud, T.C.S., Aidar, E., 1993. Salinity and temperature effects on the growth and chlorophyll-a content of some planktonic algae. Bol. Inst. Oceanogr. 41, 95–103. http://dx.doi.org/10.1590/S0373-55241993000100008.

Strasser, R.J., Srivastava, A., Tsimilli-Michael, M., 2000. The fluorescence transient as a tool to characterize and screen photosynthetic samples. Probing Photosynth. Mech. Regul. Adapt. 443–480 ISBN 9780748408214.

Svensson, F., Norberg, J., Snoeijs, P., 2014. Diatom cell size, coloniality and motility: trade-offs between temperature, salinity and nutrient supply with climate change. PLoS One 9. http://dx.doi.org/10.1371/journal.pone.0109993.

Thompson, P.A., Guo, M.X., Harrison, P.J., Whyte, J.N.C., 1992. Effects of variation in temperature. II. On the fatty-acid composition of eight species of marine phytoplankton. J. Phycol. 28, 488–497. http://dx.doi.org/10.1111/j.0022-3646.1992.00488.x.

Ukeles, R., 1958. The effect of temperature on the gorwth and survival of several marine algal species. Algae 120, 255–264. http://dx.doi.org/10.2307/1539381.

Upchurch, R.G., 2008. Fatty acid unsaturation, mobilization, and regulation in the response of plants to stress. Biotechnol. Lett. 30, 967–977. http://dx.doi.org/10.1007/s10529-008-9639-z.

Vagner, M., Lacoue-Labarthe, T., Zambonino Infante, J.-L., Mazurais, D., Dubillot, E., Le Delliou, H., Quazuguel, P., Lefrançois, C., 2015. Depletion of essential fatty acids in the food source affects aerobic capacities of the golden grey mullet liza aurata in a warming seawater context. PLoS One 10, 1–19. http://dx.doi.org/10.1371/journal.pone.0126489.

Wernberg, T., Smale, D.A., Tuya, F., Thomsen, M.S., Langlois, T.J., de Bettignies, T., Bennett, S., Rousseaux, C.S., 2012. An extreme climatic event alters marine ecosystem structure in a global biodiversity hotspot (supplementary information). Nat. Clim. Change 3, 78–82. http://dx.doi.org/10.1038/nclimate1627.

Wiktorowska-Owczarek, A., Berezinska, M., Nowak, J.Z., 2015. PUFAs: structures, metabolism and functions. Adv. Clin. Exp. Med. 24, 931–941. http://dx.doi.org/10.17219/acem/31243.

Xia, S., Wang, K., Wan, L., Li, A., Hu, Q., Zhang, C., 2013. Production, characterization, and antioxidant activity of fucoxanthin from the marine diatom *odontella aurita*. Mar. Drugs 11, 2667–2681. http://dx.doi.org/10.3390/md11072667.

Xu, Z., Jiang, Y., Zhou, G., 2015. Response and adaptation of photosynthesis, respiration, and antioxidant systems to elevated CO2 with environmental stress in plants. Front. Plant Sci. 6, 1–17. http://dx.doi.org/10.3389/fpls.2015.00701.

Yongmanitchai, W., Ward, O.P., 1991. Growth of and omega-3 fatty acid production by *Phaeodactylum tricornutum* under different culture conditions. Appl. Environ. Microbiol. 57, 419–425 PMID: 2014989.

Zhao, P., Gu, W., Wu, S., Huang, A., He, L., Xie, X., Gao, S., Zhang, B., Niu, J., Lin, a P., Wang, G., 2014. Silicon enhances the growth of *Phaeodactylum tricornutum* Bohlin under green light and low temperature. Sci. Rep. 4, 3958. http://dx.doi.org/10.1038/srep03958.

Zhu, X.G., Govindjee, Bake, N.R., DeSturler, E., Ort, D.R., Long, S.P., 2005. Chlorophyll a fluorescence induction kinetics in leaves predicted from a model describing each discrete step of excitation energy and electron transfer associated with photosystem II. Planta 223, 114–133. http://dx.doi.org/10.1007/s00425-005-0064-4.